

4 17060
OBSERVATIONS, CHIEFLY BY THE DEGENERATION
METHOD, ON POSSIBLE EFFERENT FIBRES IN
THE DORSAL NERVE-ROOTS OF THE TOAD AND
FROG. BY H. H. DALE, B.A.

Reprinted from the Journal of Physiology.
Vol. XXVII. Nos. 4 & 5, December 23, 1901.

[Reprinted from the *Journal of Physiology*.
Vol. XXVII. No. 4 & 5, December 23, 1901.]

WELLCOME	
LIBRARY	
Coll.	Wellcome
Coll.	pam
No.	WL100
	1901
	D130



22501274983



OBSERVATIONS, CHIEFLY BY THE DEGENERATION METHOD, ON POSSIBLE EFFERENT FIBRES IN THE DORSAL NERVE-ROOTS OF THE TOAD AND FROG. By H. H. DALE, B.A., *formerly Scholar of Trinity College, Cambridge, and Coutts Trotter Student.*

EXPERIMENTS made with the object of determining whether efferent fibres exist in the dorsal spinal nerve-roots of the frog have given contradictory results in the hands of different observers. On the one hand Steinach ⁽¹⁾ finds that spinal nerves send visceromotor fibres to the œsophagus, stomach and intestine solely by the dorsal roots, and to the rectum and bladder in part by the dorsal roots. On the other hand, Horton-Smith ⁽²⁾ found no evidence for the presence of visceromotor fibres in the dorsal roots: but in a small percentage of cases he found that motor fibres to certain skeletal muscles were contained in the 7th, 8th or 9th dorsal root of one side. Wana ⁽³⁾, working under Steinach, has been able to observe this effect on skeletal muscles, though the number of cases in which it occurred was relatively smaller than in Horton-Smith's experiments.

The above observations were all made by the method of stimulation, and it seemed better to attack the question by another method of experiment than simply to add to the tale of conflicting evidence. The presumption being that any departure from the Bell-Magendie law of the conduction of impulses will be associated with a corresponding exception to the Wallerian law of degeneration, I have given most attention to the degeneration method, though some experiments have also been made by the old method of stimulation. The results on certain points seem to be decisive, but I have been unable, for lack of time, to carry out the observations as fully as I had hoped.

DEGENERATION EXPERIMENTS.

The main object of my experiments was to determine whether, on section of the dorsal roots of an Amphibian, the degeneration of the fibres follows Waller's law or not.

Method of operation. All my observations have been made upon the common toad, *Bufo vulgaris*, which, on account of its thicker skin and less spacious dorsal lymph-sac, its ready recovery from large doses of ether, and the ease with which it can be kept in a clean dry chamber, forms a far better subject for operation than the frog. The toad was washed thoroughly with soap and water and was then anaesthetised by placing it in a beaker containing 150 c.c. of water to which 3 c.c. of ether had been added. In this it was left until the limbs became perfectly flaccid, respiration ceased, the mouth fell open, and no trace of corneal reflex remained. When anaesthesia is carried to this extent there is very little bleeding on opening up the spinal canal.

The dorsal skin of the anaesthetised animal was washed with corrosive sublimate ($\frac{1}{500}$) and then with sterilised 0.6% saline. The latter only was used as lotion after the first incision.

A longitudinal median cut was made through the dorsal skin, extending from a short way behind the last to about the 5th vertebra. The fascia dorsalis was divided along each side of the spinous processes of the last 4 vertebrae, the tendon of origin of the longissimus dorsi on each side removed from the urostyle, and the muscles turned forwards to their insertion. The dorsal surface of the neural arches of the 6th, 7th, 8th and 9th vertebrae was thus exposed. The animal was then held firmly with the left hand while, with fine scissors, the intercrural and interspinal muscles and ligaments were divided between the 7th and 8th vertebrae. With strong scissors the laminae of the neural arch of either the 7th or 8th vertebra were then cut through on each side of the middle line. During this part of the operation it was necessary to hold the whole animal in the left hand, with which the skin could be drawn away from the cut by pressure on the flank, and the spine slightly flexed by depression of the head. No method of fixing the animal approached this in convenience. The hand was carefully washed in corrosive sublimate before further procedure. The detached middle of the neural arch could now be raised and turned forwards or backwards. A probe was then passed into the spinal canal, the bundle of long roots raised and several roots cut across with fine scissors. The roots were returned to the spinal canal, bleeding stopped by pads of cotton-wool soaked in sterilised saline, the dorsum of the vertebra replaced, the longissimus dorsi muscles restored to their normal position, the dorsal lymph-sac freed from blood-clots by saline pads, and the dorsal wound stitched with sterilised silk ligatures. The whole operation was completed with ease in from 10 to 15 minutes, during which

time the animal remained completely under the influence of the anæsthetic.

The animal was usually kept for a day or two in a perforated pan of enamelled iron under a bell-glass and then transferred to the vivarium. Of 7 toads on which the operation was performed 3 died within a week, for no reason which could be ascertained. The animal in each of these cases became more and more sluggish, finally dying in the night. Of the other four, one, in which most of the sciatic roots were cut, died after 17 days; another lived for 30 days; the third was killed 37 days, the fourth 49 days after the operation, by which time the dorsal wound had healed completely.

Several frogs were operated upon in the same manner, but none lived long enough to give the required results. The animal appears to be much more sensitive to the effect of ether, and the looseness and delicacy of the skin makes difficult the accurate closure of a wound like that needed for the operation. Moreover the median position of many of the dorsal cutaneous nerves renders their division unavoidable. This is not so in the case of the toad, and may be one reason for the far readier healing observed in the latter.

Results of experiments. The following are the details of the effects of operation as determined by dissection of the 4 toads in which it was successfully performed.

No. of experiment	No. of days of degeneration	Roots divided, as determined by dissection			
		Left side		Right side	
		Dorsal	Ventral	Dorsal	Ventral
1	17	8th and 9th	7th, 8th, 9th	9th	8th and 9th
2	30	9th	9th	0	0
3	37	9th	8th, 9th, 10th	9th and 10th	10th
4	49	8th, 9th, 10th	9th	9th	0

The method of examination adopted was the same in all cases. The central and peripheral ends of the divided dorsal roots were cut out, carefully extended on small slips of filter-paper, treated with 1% osmic acid for 12 hours, washed for 24 hours in running water, and then teased in short segments, in dilute glycerine. The teased preparations were exhaustively examined with a magnifying power of about 400.

In the peripheral cut ends of the dorsal roots not a single fibre was discovered showing any sign of degeneration.

In the central cut ends the fibres had all undergone degeneration except in the case of one root—the 9th dorsal root of the right side in Exp. No. 4. In No. 1 degeneration had not proceeded far, and lengths

of apparently sound fibre were here and there discoverable; but, when traced through the preparation, these were in all cases found to be continuous with degenerate portions. In the other experiments degeneration had proceeded so far that there was never any doubt as to the degenerated condition of the fibres.

In the central end of the 9th right dorsal root in Exp. 4, 18 fibres were found which were quite free from degeneration and could be traced as unquestionably sound fibres through every section of the root examined. These were drawn under a high power ($\frac{400}{1}$) with a camera lucida, measured and counted, with the following result:

Fibres 10—15 μ in diameter	...	5
„ about 10 μ „	...	8
„ 5—10 μ „	...	5
		18

None of the fibres, then, were of a diameter less than 5 μ , and most of them were of considerably greater diameter.

I have examined the rami of the sympathetic system, and the branches running off from the sciatic plexus to the rectum and the bladder. In none of these was any fibre found of a greater diameter than 5 μ , and the proportion of fibres attaining that size was small: that is to say, viscero-motor preganglionic fibres in the toad are never more, and mostly less than 5 μ in diameter. It is quite clear, then, that the above sound fibres cannot be of this nature. Further, the absence of corresponding degenerated fibres in the peripheral cut end of the root is a discrepancy which forbids the conclusion that these fibres were dorsal-root fibres having their trophic centre in the spinal cord and degenerating peripherally. Examination of the corresponding ventral root gave what is probably the correct explanation of their presence. This root was found to have been injured at a point near to the level of section of the dorsal root, though it was not itself divided. In the portion of the ventral root peripheral to this point of injury a number of degenerated fibres were found. It seems most probable that a small bundle of fibres was torn across and hooked up from the ventral root by the fine probe with which the dorsal root was lifted for division, and that this fine bundle remained attached to the central cut end of the dorsal root and was removed with it for examination.

Apart from this merely apparent discrepancy it is clear that the medullated fibres of the dorsal roots examined—the 7th, 8th, 9th and 10th—in the toad all follow the Wallerian law of degeneration.

The possibility that efferent fibres of non-medullated nature exist in the dorsal roots was taken into account. Numerous portions of the fresh roots were teased in 0.6% saline containing 0.04% of methylene blue, left for 30 minutes in a moist chamber and carefully examined with low and high powers of the microscope. No non-medullated fibres could be demonstrated in them, though they were well shown in control preparations made at the same time and by the same method from the splanchnic nerves.

The evidence is therefore complete that, in those dorsal roots of the toad which have been examined, there are no fibres which have their trophic centre in the spinal cord.

In other words, if stimulation of the peripheral end of these dorsal roots does give rise to visceral effects, it does not do so by the stimulation of efferent fibres in the roots—the term efferent fibres being used in the accepted sense. Moreover, since it is most improbable that, in such a fundamental physiological character the frog should differ from the toad, I think the same conclusion holds for the frog.

There remains the possibility that the effect described by Steinach is produced by stimulation of the afferent and not of the efferent fibres. Bayliss⁽⁶⁾ suggests that Steinach's effects may be due to "antidromic" impulses of the kind which he himself demonstrated on excitation of dorsal roots in the dog. This possibility drives us back to the method of stimulation.

NOTE ON STIMULATION EXPERIMENTS.

A few observations were made by the old method of stimulation on *Rana temporaria* and *Bufo vulgaris*.

In *Rana* I obtained the contraction of certain skeletal muscles, described by Horton-Smith, on peripheral stimulation of a dorsal root, in 3 out of 12 cases. In each case the effect was obtained from the 8th dorsal root of one side only, the affected muscles being the semimembranosus and rectus internus minor. The contraction was unaffected by extirpation of the cord and of the ventral roots, but was abolished by pinching the dorsal root peripherally to the point of stimulation. The effect was obtained with weak faradisation (secondary coil at 24 cm., 1 Daniell) and with mechanical stimuli. I have not obtained any trace of this effect in 10 experiments made upon *Bufo*.

Neither in *Rana* nor in *Bufo* could I observe any visceromotor effects on peripheral stimulation of the 6th, 7th, 8th, 9th and 10th

dorsal roots. The secondary coil was pushed up to 10 cm. without any perceptible effect on the bladder or rectum: but as soon as some of the current was allowed to escape on to the bundle of ventral roots, as witnessed by twitching of the skeletal muscles, the bladder contracted sharply and slow contraction of both coats of the rectum appeared.

In the rectum of the toad autoperistalsis was so vigorous in all cases observed that it was impossible to be certain as to the presence of small effects. Only in the case of the 9th ventral root was the response to stimulation unmistakable, a steady contraction of both muscular coats resulting, so that the viscus became much shortened while at the same time the lumen was nearly obliterated by circular constriction.

Contractions of the bladder, on the other hand, were readily observed, the shrinking and rugosity caused by intrinsic contraction being very easily distinguishable from movements of the whole organ caused by twitching of the levator ani. The origin of the motor fibres to the bladder of the toad was found to be identical with that observed by Horton-Smith in *Rana temporaria*—stimulation of the 7th, 9th and 10th ventral roots causing contraction, while stimulation of the 8th was without effect on the bladder. The most marked effect was obtained from the 9th root, the feeblest stimulation of which caused contraction. In one case the mere lifting of this root to lay it upon the electrodes was regularly attended by obvious contraction of the bladder. This result differs from Steinach's observation on the innervation of the bladder in *Rana esculenta*, not only in my failure to obtain any effect from the dorsal roots but in the arrangement of the outflow in the ventral roots. Steinach obtained contraction of the bladder on stimulating the 7th, 8th and 9th dorsal and ventral roots.

I am indebted to Dr Langley for much kind assistance and advice.

BIBLIOGRAPHY.

- (1) Steinach. Pflüger's Archiv, LX. p. 593, 1895; LXXI. p. 523, 1898.
- (2) Horton-Smith. This Journal, XXI. p. 101, 1897.
- (3) Wana. Pflüger's Archiv, LXXI. p. 555, 1898.
- (4) Waters. This Journal, VI. p. 460, 1885.
- (5) Bayliss. This Journal, XXVI. p. 173, 1901.

